immunocompromised due to infection with an immunodeficiency virus.

10.(amended) The method according to [claim 7] **claim 1** wherein the mammal is immunocompromised due to radiation therapy.

11.(amended) The method according to [claim 7] **claim 1** wherein the mammal is immunocompromised due to chemotherapy.

12.(amended) The method according to [claim 7] **claim 1** wherein the mammal is selected from the group consisting of humans, mice, scid/scid mice, SCID-hu mice, and CID horses.

14.(amended) The method according to [claim 7] **claim 1** wherein the mammal is transplanted with non-autologous hematopoietic tissue.

17.(amended) The method according to [claim 15] **claim 2** wherein the mammal is a human [and the non-autologous hematopoietic cells are injected].

18.(Amended) A method of treating an immunocompromised mammal comprising administering to the **[animal] mammal** an effective amount of non-autologous hematopoietic cells **wherein the mammal is selected from the group consisting of human, mice, SCID/SCID mice, SCID-hu Thy/Liv mice and CID horses,** and administering to the mammal an effective amount of an agent which selectively decreases the number of endogenous macrophages to a level sufficient to reduce depletion of the non-autologous hematopoietic cells.

19.(amended) A non-human **immunocompromised** mammal comprising human hematopoietic cells wherein the mammal contains a decreased level of endogenous macrophages sufficient to reduce depletion of non-autologous hematopoietic cells wherein the decreased level of endogenous macrophages is achieved by administering to the mammal an effective amount of an agent which selectively decreases the number of endogenous macrophages.

28.(Amended) The method according to claim 26 wherein the blood **[cells.] cells** are CD4+ T cells.

31.(Amended) A method of improving engraftment efficiency for transplantation of a population of non-autologous hematopoietic stem cells in a host mammal having an endogenous hematopoietic stem cell population, comprising the steps of ablating the endogenous hematopoietic stem cell population of the mammal and transplanting the non-autologous hematopoietic stem cells into the host **[animal] mammal** in conjunction with administering to the mammal an effective amount of an agent which selectively decreases the **[numer] number** of endogenous macrophages in the host mammal.

Please add the following new claims:

36. A method of reducing depletion of non-autologous hematopoietic cells in an immunocompromised mammal comprising,

a) administering to the mammal an effective amount of non-autologous hematopoietic cells wherein the mammal is selected from the group consisting of human, mice, SCID/SCID mice, SCID-hu Thy/Liv mice and CID horses, and

b) administering to the mammal an effective amount of an agent selected from the group consisting of dichloromethylene diphosphonate, L-leucine methyl ester, and colloidal carbon which selectively decreases the number of endogenous macrophages to a level effective to reduce depletion of the non-autologous hematopoietic cells.

37. The method according to claim 36 wherein the mammal is SCID-hu Thy/Liv mice.

38. The method according to claim 36 wherein the agent is dichloromethylene diphosphonate.

39. The method according to claim 38 wherein the dichloromethylene diphosphonate is liposome-encapsulated.

40. The method of according to claim 1 wherein the agent is selected from the group consisting of dichloromethylene diphosphonate, L-leucine methyl ester and colloidal carbon.

Please cancel claims 7, 15, 16 and 20.

REMARKS

Claims 1- 3, 5, 8 – 14, and 16 – 40 are pending in this application. Applicants have added new claims 36 – 40, and support can be found in the original claims, at page 7, lines 13 - 16, and page 10, lines 17 – 30 of the disclosure. Claims 7, 15, 16 and 20 have been canceled.

Pending claims 1 – 3, 5, 8 – 14, 17 - 18, 28 and 31 are rejected under 35 U.S.C. §112, second paragraph and pending claims 1 – 3, 5, 8 – 14, 17 - 19 and 21 – 35 are rejected under 35 U.S.C. §112, first paragraph. As indicated by the Examiner, claims 1 – 3, 5, 8 – 14, 17 – 19 and 21 - 35 are free of the prior art, and the previous rejections under 35 U.S.C. §103 have been withdrawn. In view of the following remarks, Applicants respectfully request the Examiner to reconsider and withdraw all rejections under 35 U.S.C. §112.

Applicants have amended claims 1, 3, 8, 10 – 12, 14, 17 -19, 28 and 31 to more particularly point out and distinctively claim the instant invention. New matter has not been added by the

amendment. Specifically, claim 1 now recites a step directed to "administering the non-autologous hematopoietic cells to an immunocompromised mammal". As suggested by the Examiner, the phrase "non-autologous hematopoietic" has been included in front of the term "cells" in claim 3. Claim dependency has been changed for claims 8, 10 – 12, 14, and 17. The term "animal" on line 2 of claim 18 has been replaced with the term "mammal" and the mammal is defined as consisting of human, mice, SCID/SCID mice, SCID-hu Thy/Liv mice or CID horses. The term immunocompromised has been included in claim 19. The period after the term "cells" first occurrence has been deleted in claim 28. The term "host animal" of claim 31, line 5 has been replaced with the term "mammal", and the correct spelling for number has been provided in place of "numer".

Applicants submit the amendment to the claims should render moot the rejections under 35 U.S.C. §112, second paragraph.

With respect to the rejection under 35 U.S.C. §112, first paragraph, the Examiner has alleged that the specification does not reasonably provide enablement for,

> a method of reducing depletion in **any and all mammals** of non-autologous hematopoietic cells comprising administering to the mammal an effective amount of **any agent** which selectively decreases the number of endogenous macrophages to a level effective to reduce depletion of non-autologous hematopoietic cells (or methods of restoring or improving engraftment efficiency for transplantation comprising the same methodology – claims 24 and 31).

Applicants contend that the specification is enabled for the claimed methods wherein the host mammal is an immunocompromised mammal and that the claims should not be limited to specific mammals. Applicants' specification states at page 7,

> "The methods are suitable for use in immunocompromised animals to prolong survival of non-autologous hematopoietic cells. Suitable immunocompromised animals include, but are not limited to, humans, SCID mice, SCID-hu mice, CID horse and transgenic immunodeficient mice."

Specific examples of immunodeficient mice and immunocompromised humans are provided. Preferred mammals are listed as human, mice, SCID/SCID mice, SCID-hu Thy/Liv mice or CID horses. While the specification details the use of the claimed invention in SCID mice (Examples 1 – 3, 6) and SCID-hu Thy/Liv mice (Examples 4 – 5, 7 – 9), the specification in combination with information known in the art teach how to make and use the invention without undue experimentation.

Applicants further contend effective doses of a therapeutically effective agent (particularly dichloromethylene diphosphonate - DMDP) are capable of extrapolation from the SCID-hu mouse model to humans.

At page 8, it is disclosed the claimed methods are suitable for use in humans and particularly those infected with HIV who lack certain subsets of PBLs such as CD4+ lymphocytes. It is taught that an effective amount of human hematopoietic blood cells can be administered (intravenously) to the patient in conjunction with decreasing the endogenous macrophages. The SCID-hu mouse was known at the time of filing the present application as a well-accepted model for human disease. Additionally, DMDP had been used in humans at the time the invention was made. At page 19 it is taught,

> the effective amount of the agent ..... In the case of liposome encapsulated Cl_2MDP the effective concentration in mice is in the range of 0.005 to 0.010 ml of liposomes (containing 23.5 mg/ml lipid per 10 to 15 mM Cl_2MDP) per gram of mouse weight. While extrapolation to humans is not directly proportional, typically, the effective range would be 5 to 10 ml of these liposomes per kg of human weight.

With respect to the "agent", at page 10 of the disclosure it is stated that "decreasing the number of endogenous macrophages can be done by any method known in the art. Preferably, the macrophages are decreased by administration of an agent which selectively kills macrophages. For instance, see, Van Rooijen and Claasen (1988). More preferably, Cl_2MDP is administered in a manner whereby it is taken up by macrophages but not other cells types. Additionally at page 7 it is stated that endogenous macrophages can be depleted by treatment with L-leucine methyl ester, and by the administration of colloidal carbon to the reticuloendothelial systems.

The Examiner has also stated that while the specification is enabling for a method of reducing the depletion of non-autologous hematopoietic cells in a mammal lacking functional endogenous B- and T cells, there is no support or evidence within the specification that the elimination of endogenous macrophages improves tolerance to non-autologous hematopoietic cells in mammals which have functional T-cells and B-cells. Applicants believe inclusion of the term immunocompromised to the claims addresses this issue..

Applicants submit the rejections under 35 U.S.C. §112 1st and 2nd paragraph should be withdrawn in view of the amendment and comments presented herein. All claims are in form for allowance and allowance is kindly solicited. A clean copy of the pending claims is attached hereto for the Examiner's convenience.

Respectfully submitted,

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Date: 2/10/99

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CLEAN COPY OF PENDING CLAIMS:

1. A method of reducing depletion in a mammal of non-autologous hematopoietic cells comprising, administering to an immunocompromised mammal an effective amount of non-autologous hematopoietic cells, and administering to the mammal an effective amount of an agent which selectively decreases the number of endogenous macrophages to a level effective to reduce depletion of the non-autologous hematopoietic cells.

2. The method according to claim 1 wherein the non-autologous hematopoietic cells are injected into the mammal.

3. The method according to claim 1 wherein the non-autologous hematopoietic cells are made by hematopoietic tissue engrafted into the mammal.

5. The method according to claim 1 wherein the agent is liposome-encapsulated dichloromethylene diphosphonate.

8. The method according to claim 1 wherein the mammal is immunocompromised due to infection with an immunodeficiency virus.

9. The method according to claim 8 wherein the mammal is human and the virus is human immunodeficiency virus.

10. The method according to claim 1 wherein the mammal is immunocompromised due to radiation therapy.

11. The method according to claim 1 wherein the mammal is immunocompromised due to chemotherapy.

12. The method according to claim 1 wherein the mammal is selected from the group consisting of humans, mice, scid/scid mice, SCID-hu mice, and CID horses.

13. The method according to claim 12 wherein the mammal is a SCID-hu Thy/Liv mouse.

14. The method according to claim 1 wherein the mammal is transplanted with non-autologous hematopoietic tissue.

17. The method according to claim 2 wherein the mammal is a human.

18. A method of treating an immunocompromised mammal comprising administering to the mammal an effective amount of non-autologous hematopoietic cells wherein the mammal is

selected from the group consisting of human, mice, SCID/SCID mice, SCID-hu Thy/Liv mice and CID horses, and administering to the mammal an effective amount of an agent which selectively decreases the number of endogenous macrophages to a level sufficient to reduce depletion of the non-autologous hematopoietic cells.

19. A non-human immunocompromised mammal comprising human hematopoietic cells wherein the mammal contains a decreased level of endogenous macrophages sufficient to reduce depletion of non-autologous hematopoietic cells wherein the decreased level of endogenous macrophages is achieved by administering to the mammal an effective amount of an agent which selectively decreases the number of endogenous macrophages.

21. The non-human mammal according to claim 19 wherein the mammal contains engrafted human hematopoietic tissue.

22. The non-human mammal according to claim 19 wherein the non-autologous hematopoietic cells are produced by the engrafted tissue.

23. The mammal according to claim 19 wherein the mammal is selected from the group consisting of SCID/SCID mice, SCID-hu Thy/Liv mice and CID horses.

24. A method of restoring hematopoietic cells to an immunocompromised human comprising the steps of administering an effective amount of human peripheral blood cells in conjunction with administering to the human an effective amount of an agent which selectively decrease the number of endogenous macrophages.

25. The method according to claim 24 wherein the immunocompromised human is infected with human immunodeficient virus.

26. The method according to claim 25 wherein the peripheral blood cells are hematolymphoid.

27. The method according to claim 26 wherein the blood cells are T cells.

28. The method according to claim 26 wherein the blood cells are $CD4^+$ T cells.

29. The method according to claim 25 wherein the peripheral blood cells are administered by direct injection into the blood stream of the human.

30. The method according to claim 25 wherein the peripheral blood cells are administered by bone marrow transplantation of hematopoietic stem cells into the human.

31. A method of improving engraftment efficiency for transplantation of a population of

non-autologous hematopoietic stem cells in a host mammal having an endogenous hematopoietic stem cell population, comprising the steps of ablating the endogenous hematopoietic stem cell population of the mammal and transplanting the non-autologous hematopoietic stem cells into the host mammal in conjunction with administering to the mammal an effective amount of an agent which selectively decreases the number of endogenous macrophages in the host mammal.

32. A method according to claim 18, wherein the agent is liposome-encapsulated dichloromethylene diphosphonate.

33. A method according to claim 19, wherein the agent is liposome-encapsulated dichloromethylene diphosphonate.

34. A method according to claim 24, wherein the agent is liposome-encapsulated dichloromethylene diphosphonate.

35. A method according to claim 31, wherein the agent is liposome-encapsulated dichloromethylene diphosphonate

36. A method of reducing depletion of non-autologous hematopoietic cells in an immunocompromised mammal comprising,

a) administering to the mammal an effective amount of non-autologous hematopoietic cells wherein the mammal is selected from the group consisting of human, mice, SCID/SCID mice, SCID-hu Thy/Liv mice and CID horses, and

b) administering to the mammal an effective amount of an agent selected from the group consisting of dichloromethylene diphosphonate, L-leucine methyl ester, and colloidal carbon which selectively decreases the number of endogenous macrophages to a level effective to reduce depletion of the non-autologous hematopoietic cells.

37. The method according to claim 36 wherein the mammal is SCID-hu Thy/Liv mice.

38. The method according to claim 36 wherein the agent is dichloromethylene diphosphonate.

39. The method according to claim 38 wherein the dichloromethylene diphosphonate is liposome-encapsulated.

40. The method of according to claim 1 wherein the agent is selected from the group consisting of dichloromethylene diphosphonate, L-leucine methyl ester and colloidal carbon.